CLAIMS

1. A purified polynucleotide which encodes a thermostable polypeptide comprising an amino acid sequence having at least 80% identity to SEQ ID NO: 26, wherein said polypeptide has at least one mutation in amino acids 738 to 767 of SEQ ID NO:26, or at a position selected from the group consisting of A331, L332, D333, Y334, S335, M470, F472, M484, W550, L332, D333, and Y334, and wherein said polypeptide has DNA polymerase activity.

2. The purified polynucleotide of Claim 1, wherein said at least one mutation is selected from the group consisting of A331T, S335N, M470K, M470R, F472Y, M484V, M484T, and W550R.

3. The purified polynucleotide of Claim 1, wherein said polypeptide has at least 90% identity to SEQ ID NO: 26.

4. The purified polynucleotide of Claim 1, wherein said polypeptide has at least 95% identity to SEQ ID NO: 26.

5. The purified polynucleotide of Claim 1, wherein said polypeptide has at least 97.5% identity to SEQ ID NO: 26.

6. The purified polynucleotide of Claim 1, wherein said polypeptide comprises at least two mutations.

7. The purified polynucleotide of Claim 1, wherein said polypeptide has an amino acid sequence selected from the group consisting of SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, and SEQ ID NO: 38.

8. The purified polynucleotide of Claim 7, wherein said polypeptide has the amino acid sequence of SEQ ID NO: 20.

9. The purified polynucleotide of Claim 7, wherein said polypeptide has the amino acid sequence of SEQ ID NO: 22.

10. The purified polynucleotide of Claim 7, wherein said polypeptide has the amino acid sequence of SEQ ID NO: 24.

11. The purified polynucleotide of Claim 7, wherein said polypeptide has the amino acid sequence of SEQ ID NO: 28.

12. The purified polynucleotide of Claim 7, wherein said polypeptide has the amino acid sequence of SEQ ID NO: 30.

13. The purified polynucleotide of Claim 7, wherein said polypeptide has the amino acid sequence of SEQ ID NO: 32.

14. The purified polynucleotide of Claim 7, wherein said polypeptide has the amino acid sequence of SEQ ID NO: 34.

15. The purified polynucleotide of Claim 7, wherein said polypeptide has the amino acid sequence of SEQ ID NO: 36.

16. The purified polynucleotide of Claim 7, wherein said polypeptide has the amino acid sequence of SEQ ID NO: 38.

17. The purified polynucleotide of Claim 1, wherein said polynucleotide has a sequence selected from the group consisting of SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, and SEQ ID NO: 37.

18. A purified polynucleotide that is complementary to the polynucleotide of Claim 1.

19. A purified polynucleotide which hybridizes under stringent conditions to the polynucleotide of Claim 1; wherein said stringent conditions comprise washing in 5X SSC at a temperature from 50 to 68°C.

20. A vector comprising the purified polynucleotide of Claim 1.

21. The vector of Claim 20, wherein said polynucleotide is operably linked to a heterologous expression sequence.

22. A host cell comprising the purified polynucleotide of Claim 1.

23. A purified thermostable polypeptide comprising an amino acid sequence having at least 80% identity to SEQ ID NO: 26, wherein said polypeptide has at least one mutation in amino acids 738 to 767 of SEQ ID NO:26, or at a position selected from the group consisting of A331, L332, D333, Y334, S335, M470, F472, M484, W550, L332, D333, and Y334, and wherein said polypeptide has DNA polymerase activity.

24. The purified polypeptide of Claim 22, wherein said at least one mutation is selected from the group consisting of A331T, S335N, M470K, M470R, F472Y, M484V, M484T, and W550R.

25. The purified polypeptide of Claim 23, wherein said polypeptide has at least 90% identity to SEQ ID NO: 26.

26. The purified polypeptide of Claim 23, wherein said polypeptide has at least 95% identity to SEQ ID NO: 26.

27. The purified polypeptide of Claim 23, wherein said polypeptide has at least 97.5% identity to SEQ ID NO: 26.

28. The purified polypeptide of Claim 23, wherein said polypeptide wherein said polypeptide comprises at least two mutations.

29. The purified polypeptide of Claim 23, wherein said polypeptide has an amino acid sequence selected from the group consisting of SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, and SEQ ID NO: 38.

30. The purified polypeptide of Claim 29, wherein said polypeptide has the amino acid sequence of SEQ ID NO: 20.

31. The purified polypeptide of Claim 29, wherein said polypeptide has the amino acid sequence of SEQ ID NO: 22.

32. The purified polypeptide of Claim 29, wherein said polypeptide has the amino acid sequence of SEQ ID NO: 24.

33. The purified polypeptide of Claim 29, wherein said polypeptide has the amino acid sequence of SEQ ID NO: 28.

34. The purified polypeptide of Claim 29, wherein said polypeptide has the amino acid sequence of SEQ ID NO: 30.

35. The purified polypeptide of Claim 29, wherein said polypeptide has the amino acid sequence of SEQ ID NO: 32.

36. The purified polypeptide of Claim 29, wherein said polypeptide has the amino acid sequence of SEQ ID NO: 34.

37. The purified polypeptide of Claim 29, wherein said polypeptide has the amino acid sequence of SEQ ID NO: 36.

38. The purified polypeptide of Claim 29, wherein said polypeptide has the amino acid sequence of SEQ ID NO: 38.

39. A kit for amplifying DNA comprising:

a purified thermostable polypeptide, wherein said polypeptide has at least 80% homology to SEQ ID NO: 26, wherein said polypeptide has at least one mutation in amino acids 738 to 767 of SEQ ID NO:26, or at a position selected from the group consisting of A331, L332, D333, Y334, S335, M470, F472, M484, W550, L332, D333, and Y334, and wherein said polypeptide has DNA polymerase activity;

a concentrated buffer solution, and optionally one or more divalent metal ions; and

a mixture of deoxyribonucleotides.

40. The kit of Claim 39, wherein said at least one mutation is selected from the group consisting of A331T, S335N, M470K, M470R, F472Y, M484V, M484T, and W550R.

41. The kit of Claim 39, wherein said divalent metal ion is Mg^{2+} or Mn^{2+}.

42. The kit of Claim 39, wherein said polypeptide has at least 90% identity to SEQ ID NO: 26.

43. The kit of Claim 39, wherein said polypeptide has at least 95% identity to SEQ ID NO: 26.

44. The kit of Claim 39, wherein said polypeptide has at least 97.5% identity to SEQ ID NO: 26.

45. The kit of Claim 39, wherein said polypeptide comprises at least two mutations.

46. The kit of Claim 39, wherein said polypeptide has an amino acid sequence selected from the group consisting of SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, and SEQ ID NO: 38.

47. The kit of Claim 46, wherein said polypeptide has the amino acid sequence of SEQ ID NO: 20.

48. The kit of Claim 46, wherein said polypeptide has the amino acid sequence of SEQ ID NO: 22.

49. The kit of Claim 46, wherein said polypeptide has the amino acid sequence of SEQ ID NO: 24.

50. The kit of Claim 46, wherein said polypeptide has the amino acid sequence of SEQ ID NO: 28.

51. The kit of Claim 46, wherein said polypeptide has the amino acid sequence of SEQ ID NO: 30.

52. The kit of Claim 46, wherein said polypeptide has the amino acid sequence of SEQ ID NO: 32.

53. The kit of Claim 46, wherein said polypeptide has the amino acid sequence of SEQ ID NO: 34.

54. The kit of Claim 46, wherein said polypeptide has the amino acid sequence of SEQ ID NO: 36.

55. The kit of Claim 46, wherein said polypeptide has the amino acid sequence of SEQ ID NO: 38.

56. The kit of Claim 39, further comprising a 5' to 3' exonuclease or a 3' to 5' exonuclease.

57. The kit of Claim 56, wherein said 5' to 3' exonuclease has SEQ ID NO: 50.

58. The kit of Claim 56, wherein said 3' to 5' exonuclease has SEQ ID NO: 51.

59. A method for reverse transcribing an RNA comprising:

a) providing a reverse transcription reaction mixture comprising said RNA, a primer, a divalent cation, and a purified thermostable polypeptide comprising an amino acid sequence having at least 80% identity to SEQ ID NO: 26, wherein said polypeptide has at least one mutation in amino acids 738 to 767 of SEQ ID NO:26, or at a position selected from the group consisting of A331, L332, D333, Y334, S335, M470, F472, M484, W550, L332, D333, and Y334, and wherein said polypeptide has DNA polymerase activity.; and

b) treating said reaction mixture at a temperature and under conditions suitable for said purified polypeptide to initiate synthesis of an extension product of said primer to provide a cDNA molecule complementary to said RNA.

60. The method of Claim 59, wherein said at least one mutation is selected from the group consisting of A331T, S335N, M470K, M470R, F472Y, M484V, M484T, and W550R.

61. A method of identifying thermostable mutant polypeptides comprising

a) packaging a vector in which a polynucleotide encoding a phage coat protein is fused to a polynucleotide encoding a protein having at least 80% identity to SEQ ID NO: 26 into a phage;

b) expressing the fusion protein;

c) isolation of phage particles;

d) infecting E. coli and incubating the infected E. coli;

e) detecting the fusion protein;

f) assessing polymerase activity.

62. The method of Claim 61, wherein (b) – (f) are repeated 0 to 25 times.

63. The method of Claim 61, wherein the phage coat protein is SEQ ID NO: 39.

64. A method of identifying thermostable mutant polypeptides having a catalytic activity comprising:

a) packaging a vector in which a gene or fragment thereof encoding variants of a catalytic domain responsible for the catalytic activity fused to a gene encoding a phage coat protein;

b) isolation and purification of phage particles;

c) heating the phage-mutant polypeptide at a temperature ranging from 50°C to 90°C for a time ranging from 30 seconds to several hours;

d) cross-linking a specific substrate with a phage particle;

e) forming a reaction product from the substrate catalyzed by the thermostable mutant protein on phage, wherein the temperature is optionally regulated to be the same or greater or lower than the temperature of (c)

f) selecting the phage particles comprising a variant nucleotidic sequence encoding for the catalytic domain responsible for the catalytic activity at the regulated temperature, by capturing the reaction product or screening for said reaction product,

g) infecting *E. coli* with the phage particles selected at step (f),

h) incubating the infected *E. coli*; and

i) assessing catalytic activity of the proteins corresponding to isolated genes.

65. The method of Claim 64, wherein the gene or fragment thereof encoding variants of a catalytic domain is directly fused to the gene encoding a phage coat protein.

66. The method of Claim 64, wherein the steps (a) to (h) are repeated 0 to 20 times.

67. The method of Claim 64, wherein the gene or fragment thereof encoding variants of a catalytic domain and the gene encoding a phage coat protein, are indirectly fused by a peptide or polypeptide linker.

68. The method of Claim 67, wherein the peptide is selected from the group consisting of:

a glycine rich linker such as $(SG_4)n$ (SEQ ID NO: 39),

a human calmodulin (SEQ ID NO: 46), and

a hexahistidine binding single chain variable fragment consisting of

(i) an anti-His Tag Antibody 3D5 Variable Heavy Chain (SEQ ID NO: 47)
(ii) a linker (SEQ ID NO: 48)
(iii) an anti-His Tag Antibody 3D5 Variable Light Chain (SEQ ID NO: 49).

69. The method of Claim 67, wherein the polypeptide linker is selected from the group consisting of:

- a protein binding the substrate at high temperature

- a catalytic domain of a 5' to 3' exonuclease

- a catalytic domain of a 3' to 5'

- a catalytic domain of Bacillus circulans cyclodextringlycosyltransferase (SEQ ID NO: 52),

- a catalytic domain of Bordetella pertussis adenylate cyclase(SEQ ID NO: 53)

- a Bacillus amyloliquefaciens serine protease subtilisin (SEQ ID NO: 54), and

- a catalytic domain of Bacillus subtilis lipase A (SEQ ID NO: 55).

70. The method of Claim 64, wherein the cross-linking between the specific substrate of the catalytic domain of the polypeptide with the phage particule is made by a cross-linking agent selected from the group consisting of a:

- maleimidyl group
- iodoacetyl group
- disulfide derivative and
- any other thermostable link.

71. The method of Claim 64, wherein the catalytic domain is the catalytic domain of an enzyme selected from the group consisting of a:

- DNA polymerase,
- alpha-amylase,
- lipase,
- protease,
- a cyclodextringlycosyltransferase, and
- an adenylate cyclase.

72. The method of Claim 64, wherein the assessment of the catalytic activity of (f) is made by means of a DNA polymerization.

73. The method of Claim 64, wherein (b) is performed after (e) or during (h).

74. The method of Claim 64, wherein the temperature in (e) is regulated to be the same or greater than the temperature of (c).

75. The method of Claim 64, wherein the temperature in (e) is regulated to be the same or less than the temperature of (c).

76. A method of obtaining a thermostable variant enzyme comprising:

a) screening enzymes expressed at the surface of phage particles and

identifying at least a thermostable variant conserving its active;

catalytic domain at regulated temperature according to the method of claim 61,

b) isolating and sequencing a DNA encoding said identified thermostable variant;

c) preparing a vector comprising the DNA of step (b);

d) transfecting or infecting cells with the vector obtained at step c);

e) expressing the thermostable variant enzyme from the cells and optionally,

f) recovering, isolating and purifying said thermostable variant enzyme expressed at step (e).

77. A method of obtaining a thermostable variant enzyme comprising:

a) screening enzymes expressed at the surface of phage particles and identifying at least a thermostable variant conserving its active;

catalytic domain at regulated temperature according to the method of claim 69,

b) isolating and sequencing a DNA encoding said identified thermostable variant;

c) preparing a vector comprising the DNA of step (b);

d) transfecting or infecting cells with the vector obtained at step c);

e) expressing the thermostable variant enzyme from the cells and optionally,

f) recovering, isolating and purifying said thermostable variant enzyme expressed at step (e).

78. An insert contained in a phage selected from the group consisting of I-3168, I-3169, I-3170, I-3171, I-3172, I-3173, I-3174, I-3175, and I-3176 deposited in CNCM on February 27, 2004 under the number.

79. A recombinant host cell comprising an insert or a polynucleotide encoding a thermostable polymerase according claim 78 .